DISTINGUISHING THE TREEFROGS *Hyla versicolor* and *Hyla chrysoscelis* IN IOWA, AND THEIR DISTRIBUTIONS

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by

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An abstract of a Thesis by

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Historically, the tetraploid and diploid species pair of treefrogs, *Hyla versicolor* and *Hyla chrysoscelis* have been mapped collectively in Iowa because no distinguishing macroscopic morphological characteristics useful in recognizing the two species had been found. The present study reports identification and separation of the species by counts of stained nucleoli and measurement of scanning electron micrographs of toepad projections. The resulting distribution correlates with spot checks of call analysis. Subsequent morphometric data confirm that the two species represent two distinct populations in Iowa. *H. chrysoscelis* is distributed sporadically though the southwestern half of Iowa while *H. versicolor* is limited to the southeastern half of the state. This study suggests a decline of these species in extreme northern Iowa.

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Introduction

The *Hyla versicolor-Hyla chrysoscelis* complex is the only known diploid-tetraploid species pair of frogs in North America (Wasserman, 1970; Dalrymple, 1993). Chromosomal analysis shows *H. versicolor,* Le conte's gray treefrog, to be tetraploid (4n=48), and *H. chrysoscelis,* Cope's gray treefrog, to be diploid (2n=24) (Wasserman, 1970). Due to morphological and ecological similarities, the two species cannot be distinguished by gross morphological characteristics (McAlpine et al., 1991). The inability to differentiate these species morphologically has resulted in a clumping of their distributions (Christiansen and Bailey, 1991; Conant and Collins, 1991). The current information on the ranges of the two species is based on call pulse rate analysis of the males. When calling sympatrically, the two species can be distinguished by the pitch and duration of their call (Jaslow and Vogt, 1977; Ralin and Selander, 1979; McAlpine et al., 1991; Dalrymple, 1993). This study uses three methods (morphology, measurement of toepad projections and counts of nucleoli) with spot checks of call analysis to distinguish the species.

It was not until 1966 that *H. versicolor* and *H. chrysoscelis* were recognized as separate species (Johnson, 1966). Both species display variation in color and markings. Background coloring may be gray, light brown, or green. The belly is usually cream color. Markings occurring in a variety of patterns, may be dark gray, olive, or brown. Mottling may have a dark gray, green, or black outline, or the outline may be absent. The concealed surfaces of the thighs are distinctly yellow which gives rise to a flash of color when jumping. Large adhesive pads are present on the ends of the toes. The toes of the front feet are unwebbed in a hand-like fashion. The rear toes have moderate

webbing. These frogs are larger than most other North American treefrog species (Conant and Collins, 1991).

Certain cells of the tetraploid species are larger than those of the diploid species and can be used to distinguish the species (Cash and Bogart, 1978; Fitzgerald et. al., 1981). Treefrogs are known to have hexagonal epithelial toepad projections (Green, 1979; Green and Carson, 1988; Hanna and Barnes, 1991), and Green (1980) found these toepad projections in *H. versicolor* to be 1.81 times greater in mean relative volume than those of *H. chrysoscelis*. This difference in size is assumed to be due to the increased amount of genetic material in the cells of the tetraploid species (Green, 1980).

The difference in the amount of genetic material can also be seen in the size of the nucleus and in the number of nucleoli present. Cash and Bogart (1978) found the nuclei of *H. versicolor* to be 2.1 times the size of *H. chrysoscelis* nuclei. Similarly, a greater number of nucleoli are also present in the cells of *H. versicolor* than in *H. chrysoscelis* reflecting the increased amount of genetic material within the cells of the tetraploid species (Cash and Bogart, 1978).

Examination at the cellular level is necessary to correctly identify the two species (Cash and Bogart, 1978; Ralin and Rogers, 1979; Fitzgerald, et al., 1981; Hillis et al., 1987; Dalrymple, 1993). The present study was conducted to verify the consistency of counts of nucleoli, measurements of toepads, calls and morphology in distinguishing the species in Iowa. Judgements are made as to which method was most effective. The resulting identifications were used to map the distributions of *H. versicolor* and *H. chrysoscelis* in Iowa.

Methods and Materials

The sample of one hundred fifty-six specimens examined were comprised of either freshly collected or were preserved specimens in the Drake University Research Collection (DURC). See Appendix A for a specimen listing and Appendix B for county boundaries and labels. Some frogs used in this study had been in storage for as many as 26 years. Specimens were initially fixed in 13% formalin and were stored in 5% formalin. Thirteen morphological measurements were made with dial calipers on all specimens: (1) snout-vent length (SVL); the distance from the tip of the snout to the posteriodorsal edge of the cloaca; (2) femur length; the distance from the lateral edge of the cloaca to the knee; (3) shank length; the distance from the knee to the articulation of the tibio-fibula with the fibulare; (4) foot length; the distance from the articulation of the tibio-fibula with the fibulare the tip of the fourth (longest) toe; (5) forearm length; the distance from the elbow to the articulation of the radio-ulna with the carpals; (6) upper arm length; the distance from the articulation of the humerus with the pectoral girdle to the elbow; (7) hand length; the distance from the articulation of the radio-ulna with the carpals to the tip of the third (longest) finger; (8) toepad width; the width of the toepad on the third finger; (9) head length; the distance from the tip of the snout to the posterior edge of the rim surrounding the tympanum; (10) eye-to-nostril-length; the distance from a naris to the anterior corner of the eye; (11) nostril-to-lip length; the vertical distance from the nostril to the edge of the upper lip; (12) tympanum diameter; the horizontal width of the tympanum; and (13) head width, the widest measurement of the head, usually the distance between the corners of the mouth (Ralin and Rogers, 1979; Mason,

1988). Bilateral measurements were made on the animal's right side. A few specimens were identified initially by call prior to preservation and others were identified in sympatric populations where they could easily be identified by call. These specimens served as reference points for comparisons with toepad and cellular characteristics. Morphological data were analyzed by t-test for two samples with equal variance and reanalyzed with ANOVA. Only adults were statistically analyzed. For the purpose of this study, individuals less than 35mm SVL were considered juveniles, and those 35 mm SVL or greater were considered adults.

The third and fourth toepads on the left front foot of 17 specimens that had been prefixed in 13% formalin and stored for varying periods in 5% formalin were dissected and prepared using standard procedure for examination on the scanning electron microscope (SEM) (Postek et al., 1980). Following dissection, specimens were dehydrated through a graduated series of alcohols. They were then saturated with amyl acetate so that liquid CO_2 could be introduced into the tissue during critical point drying. After critical point drying, the toepads were mounted on stubs, stored overnight in a desiccation jar containing Drierite (calcium carbonate), ion coated with gold/palladium for protection using a Polaron Sputter Coater (model 5100, series II), and finally were viewed at 1000X magnification on the Hitachi S-500 SEM. Fixation with glutaraldehyde was omitted due to prior fixation with formalin. Measurements of the toepad epithelial projections were made on micrographs at a magnification of 2590 times. Width measurements were made on the micrographs at the widest point on the cell to the nearest millimeter, averaged, and converted to micrometers.

Counts of nucleoli were made on silver stained slides of flattened palpebral spectacles, a transparent membrane covering the cornea (Fitzgerald, et al., 1981). The palpebral spectacles were dissected, and slides were prepared using the technique of Fernandez-Gomez et al. (1969). Their technique was modified by extending the initial fixation with 1:1 1% hydroquinone: 10% formalin to 4-6 hours and reducing the silver staining incubation time to 8-12 hours. Since this staining technique employs the same concepts as photographic development; after dissection, no metal utensils were used, and procedures were performed under darkroom conditions. Wooden tooth picks were used for handling tissues. These tissues were examined and photographed under oil emersion at 1000X. Specimens whose nuclei frequently contained three or four nucleoli were categorized as *H. versicolor*. Specimens with only one or two nucleoli in each nucleus were categorized as *H. chrysoscelis* (Cash and Bogart, 1978).

Specimens identified by counts of nucleoli were analyzed for consistency of toepad projection measurement with a t-test using pooled variance. All frogs collected for this study were preserved and catalogued into the DURC where they remain available for anyone who wishes to reexamine them. Prepared slides and photographs are stored as well and are identified by the specimen catalogue number.

Results

The counts of nucleoli in silver-stained palpebral spectacle showed an apparent natural break among the 156 specimens examined. A block of typically larger frogs had two, often three, and occasionally four nucleoli per nucleus (Fig. 1A). Frogs that were frequently somewhat smaller had only one or two, never three or four nucleoli (Fig. 1B).

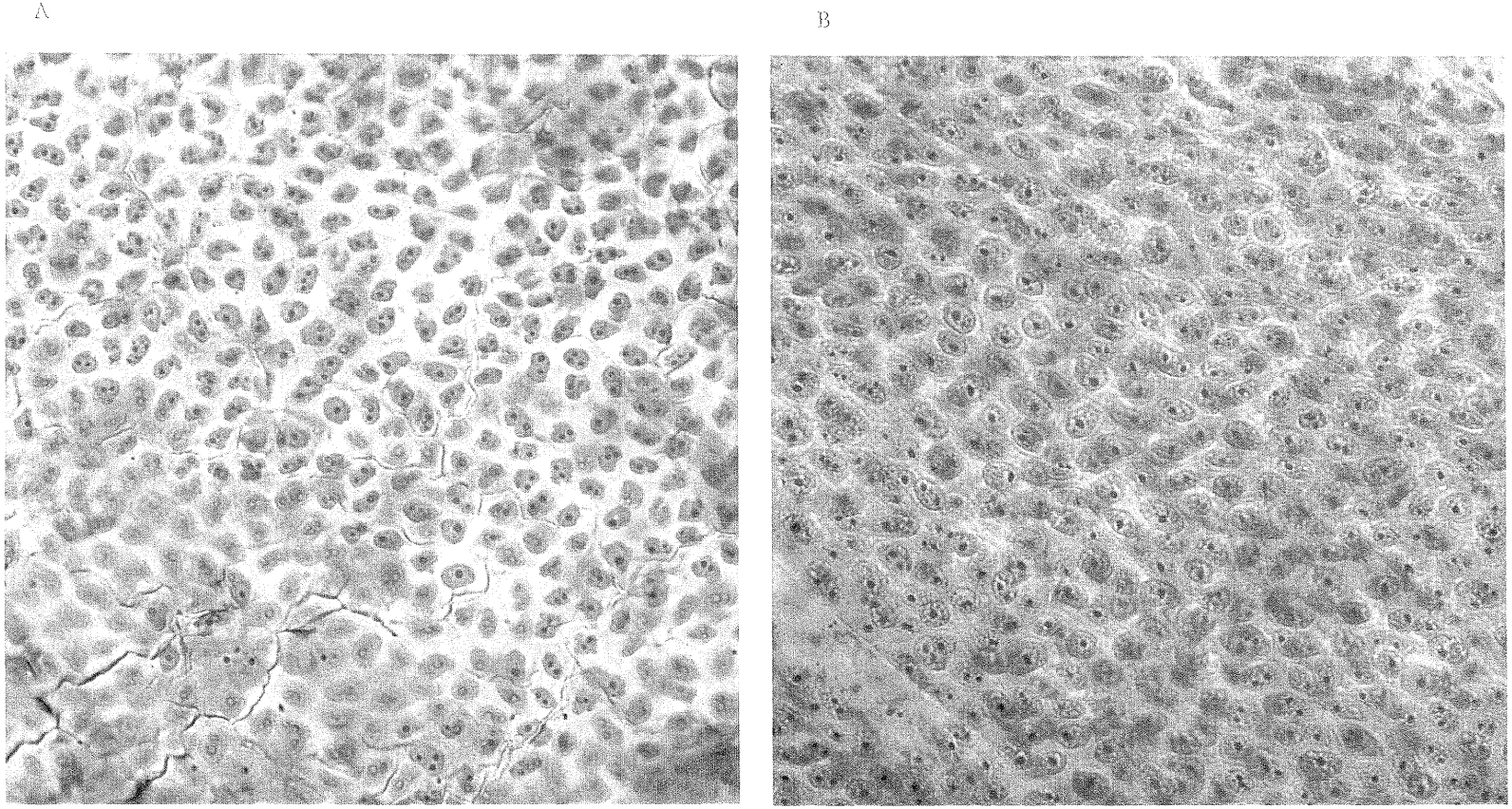


Figure 1: Micrographs are of silver stained palpebral spectacles. Cells of *H. versicolor* (A) show 3 and 4 nucleoli per nucleus. Cells showing counts of 1 or 2 sometimes have additional nucleoli out of the focal plane. *H. chrysoscelis* (B) shows counts of 1 and 2 nucleoli per cell, never more. (1000X)

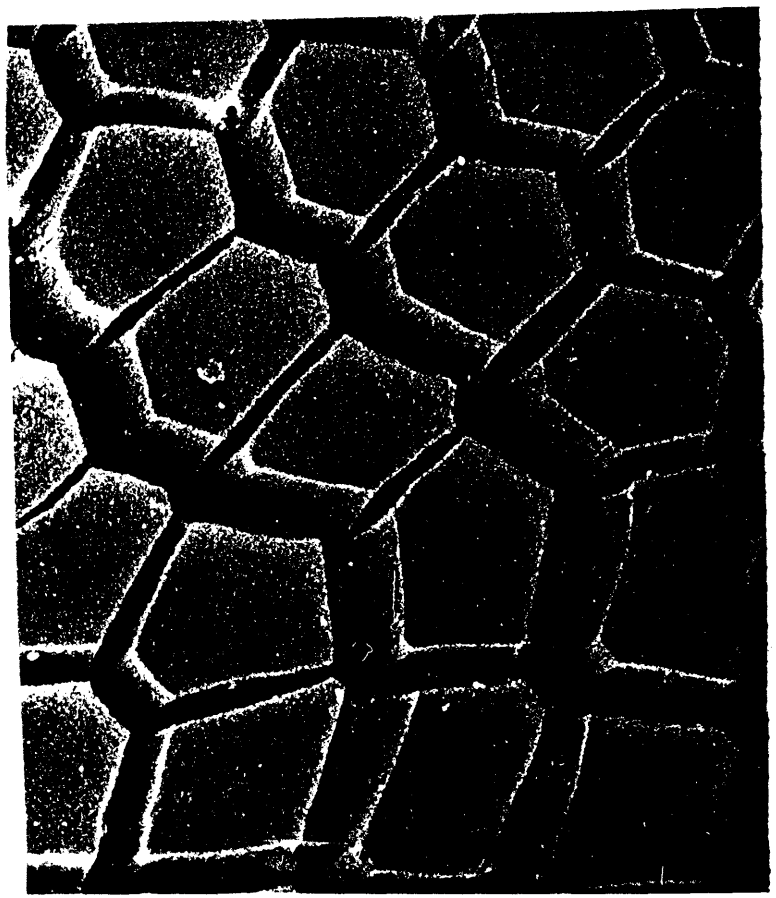
These differences were expected where the larger frogs, being tetraploid, have more DNA than the smaller diploid frogs.

Typical specimens of each species identified on the basis of number of nucleoli were compared on the basis of size of toepad projections and nature of the call to verify the value of counts of nucleoli as a primary species-identifying character in Iowa. Larger toepads were more consistently present in *H. versicolor* (Fig. 2A) than in *H. chrysoscelis* (Fig. 2B) (Table 1). It is apparent from Table 1 that many *H. chrysoscelis* from Cherokee Co. have larger toepad projections than do those from other areas. Christiansen (personal communication) observed that this population contained individuals that had *H. versicolor*-like call but no sonograms were recorded. Even with this, no *H. chrysoscelis* were found with toepad projections greater than 17μm. This is consistent with the large overlap in size-related characters examined in this study.

As a final check, calls were studied from freshly caught specimens of the two species. Typical sonograms prepared by Tim Armstrong are presented in Figures 3 and 4. Seventeen specimens of *H. versicolor* examined and ten specimens of *H. chrysoscelis* examined differed considerably in the call of longer duration with fewer cycles per second always belonging to *H. versicolor*, and the call of shorter duration with more cycles per second always belonging to *H. chrysoscelis*. Figure 5 illustrates Armstrong's mapping based on call analysis.

When studies of nucleoli, toepads and calls were complete, it was evident that two populations of gray treefrogs were present in Iowa. To determine whether these two populations in Iowa represented morphologically different animals, gross scale

A



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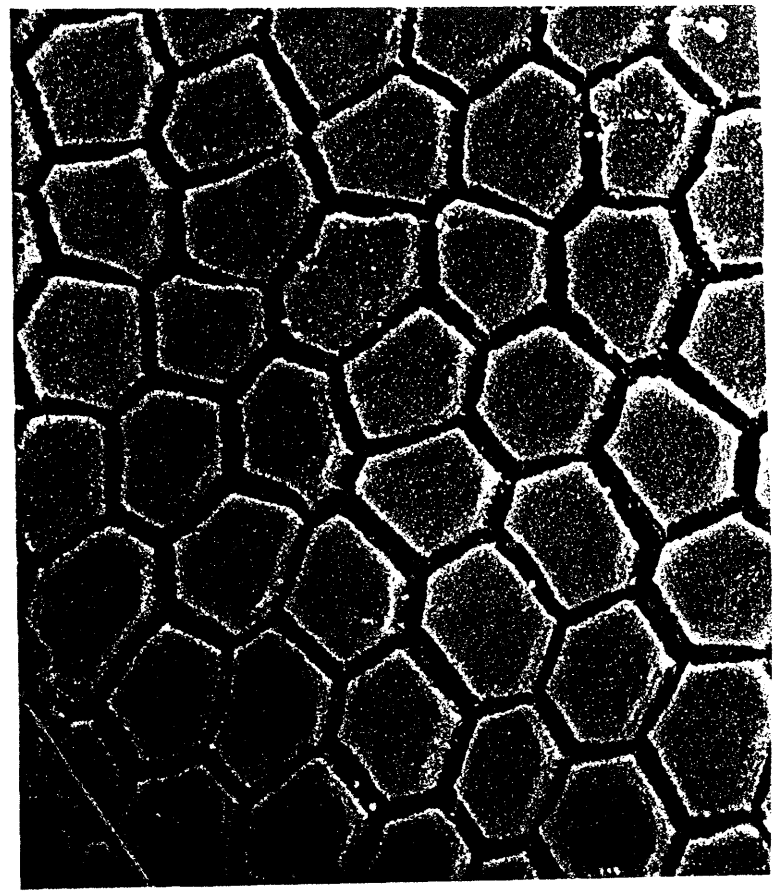

Figure 2: Scanning electron micrographs of toepad projections of *H. versicolor* (A) and *H. chyrsoscelis* (B) from Iowa. Average projection width for A is 18μm and for B is 12μm. (1554X).

Table 1: Counts of nucleoli and measurements of toepad projections. Nucleoli were silver stained using the technique by Fernandez and Gomez (1969), and toepads were measured from uniformly enlarged photomicrographs. DURC = Drake University Research Collection.

DURC Number	Counts of Nucleoli	Projection Width	Mean Width
H. versicolor			mean = 17μm
1574	3 and 4	16μm	
1570	3 and 4	16μm	
1569	3	18μm	
1568	3	18μm	
H. chrysoscelis			mean = 14.67μm
2669	1 and 2	17μm	
1611	1 and 2	12μm	
2648	1 and 2	12μm	**t-test** P = 0.016
1595	1 and 2	16μm	
1594	1 and 2	15μm	
1599	1 and 2	14μm	
1598	1 and 2	15μm	
2670	1 and 2	16μm	
2668	1 and 2	14μm	
2671	1 and 2	15μm	
1596	1 and 2	16μm	
1593	1 and 2	14μm	

A

B

Figure 3: Sonograms of call pulse frequencies of *H. versicolor* (A) and *H. chrysoscelis* (B).

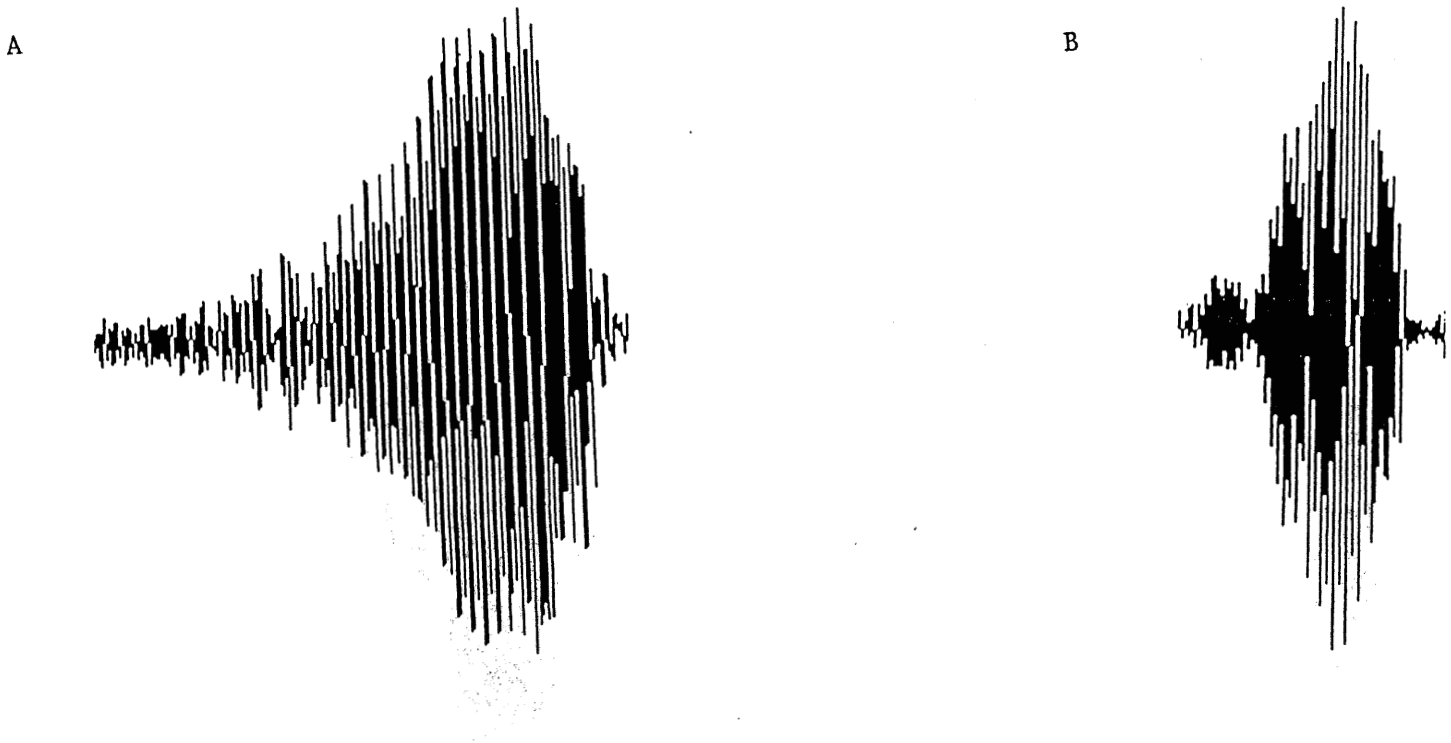

Figure 4: Sonograms of diagnostic pulse shapes for *H. versicolor* (A) and *H. chrysoscelis* (B), showing difference in calls of the two species in Iowa.

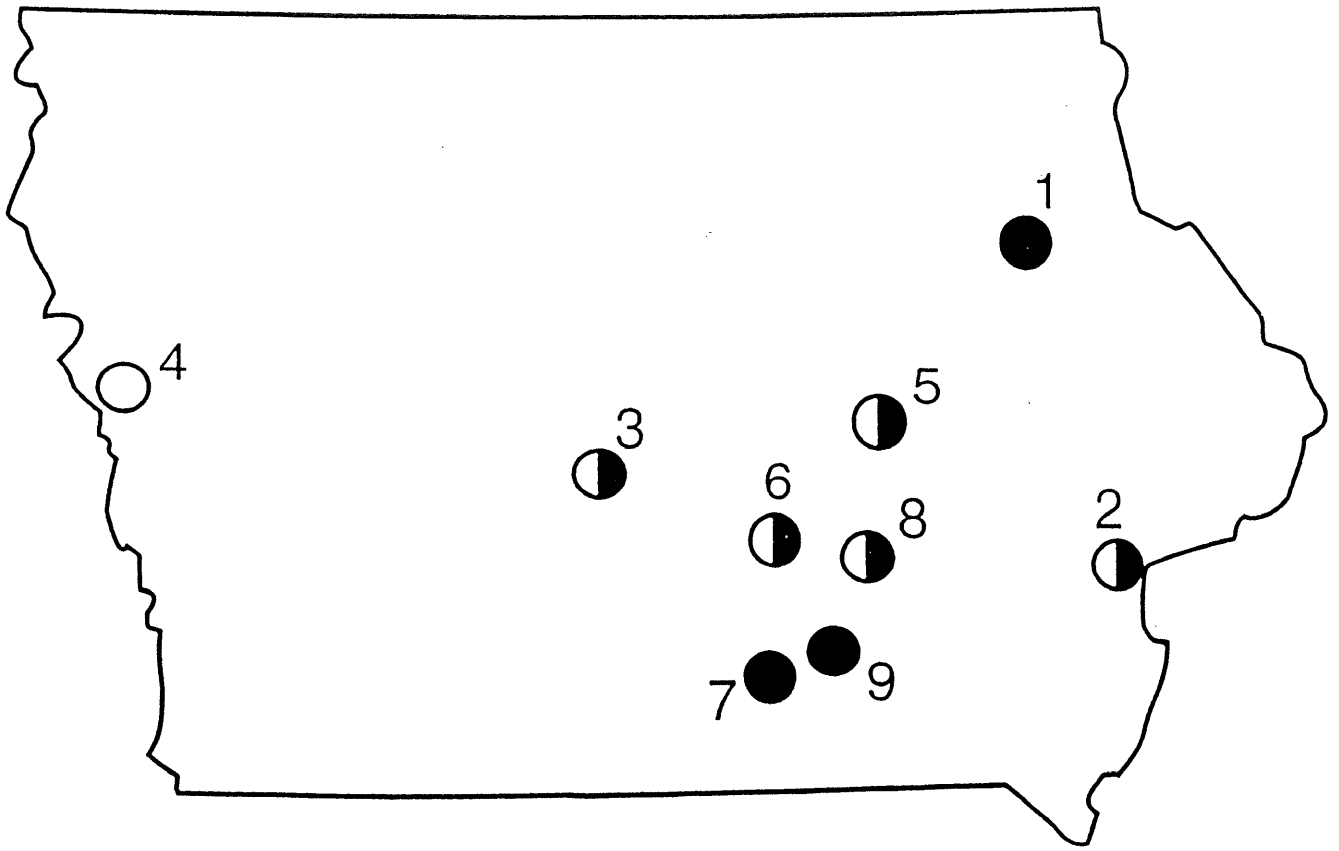

Figure 5: Distribution of *H. versicolor* (●) and *H. chrysoscelis* (○) in Iowa determined from call analysis. (Mapping provided by Tim Armstrong, Iowa State University) ◑ = both species.

measurements of all frogs greater that 35mm SVL were compared. These are shown in Table 2. While exhibiting great overlap, Table 2 shows *H. versicolor* to be significantly larger than *H. chrysoscelis* (P=.05) in 11 of 13 characters with t-tests and in seven of 13 with f-tests. Although the means of these measurements are statistically different, the raw data show complete overlap in all measurements.

With the establishment of a reliable way to distinguish *H. versicolor* and *H. chrysoscelis* in Iowa, it has become possible to plot the distribution of the two species. The DURC at this writing contains 65 specimens of *H. versicolor*. They are distributed across most of the southeastern half of the state (Fig 6). The hiatus visible in the center of that distribution is the result of failure to sample that area.

H. chrysoscelis is represented in the DURC by 91 specimens, and is distributed through most of the southwestern half of Iowa (Fig 7). Where the two species are plotted together extensive overlap exists in central and eastern Iowa with the two species collected sympatrically or nearly so in Muscatine, Louisa and Davis Counties (Fig 8). This could allow opportunity for hybridization. There is no record of hybridization of these species.

Study of rainfall distribution show the greater average yearly rainfall in southern and eastern Iowa (Fig 9). It becomes apparent when comparing the rainfall distribution with species distributions of the Gray Treefrog complex that *H. versicolor* is associated with higher rainfall areas (mean rainfall for cities examined in range = 35.2"/year). *H. chrysoscelis* exists in both high and comparably low rainfall areas (mean rainfall for cities examined in range = 32.9"/year). Two-tailed t-test comparison the five lowest

Table 2: Morphometric analysis of *H. versicolor* (*H. v.*) and *H. chrysoscelis* (*H. c.*) collected in Iowa from 1970 - 1996.

	H.c.	*H.v*	*H.c.*	*H.v*		
	Mean	Mean	Variance	Variance	t-test	f-test
SVL mm	41.92	44.91	16.10	20.56	0.0001	0.1647
femur	21.10	22.14	3.64	5.83	0.0068	0.0305
shank	19.49	20.38	3.06	4.09	0.0090	0.1226
foot	26.62	28.32	6.85	10.77	0.0013	0.0359
forearm	9.85	10.32	1.02	1.35	0.0150	0.1309
upperarm	7.74	8.34	0.97	1.17	0.0013	0.2262
hand	10.56	11.45	1.14	2.03	0.0001	0.0106
toepad	2.44	2.61	0.25	0.39	0.0902	0.0393
head (L)	13.03	13.88	1.21	2.18	0.0003	0.0097
eye-nostril	3.37	3.54	0.29	0.40	0.1108	0.1067
nostril-lip	3.36	3.73	0.26	0.31	0.0001	0.2465
tympanum	3.11	3.30	0.13	0.22	0.0091	0.0202
head (W)	14.88	16.07	2.17	4.72	0.0003	0.0010

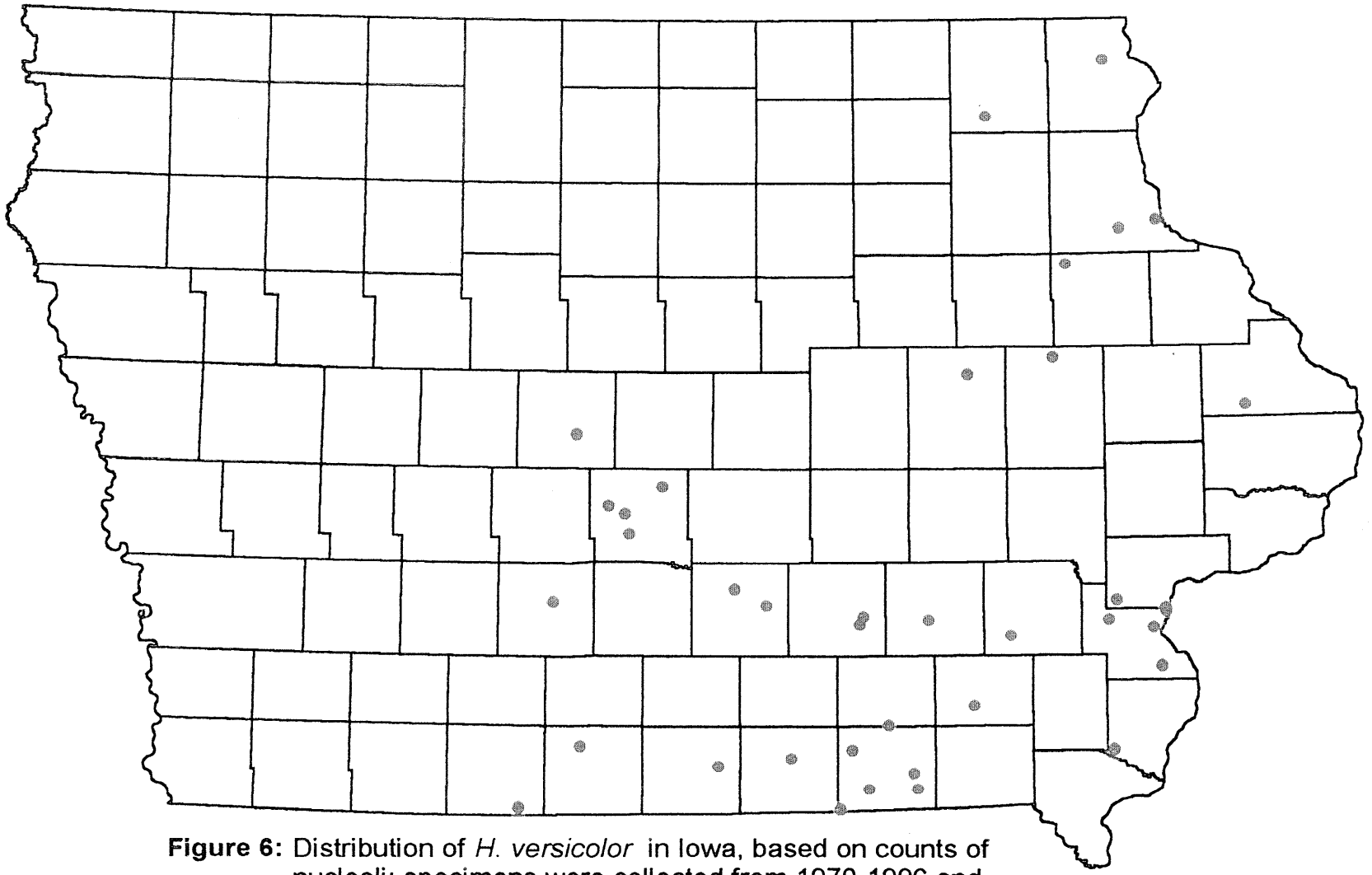

Figure 6: Distribution of *H. versicolor* in Iowa, based on counts of nucleoli: specimens were collected from 1970-1996 and are available in the Drake University Research Collection.

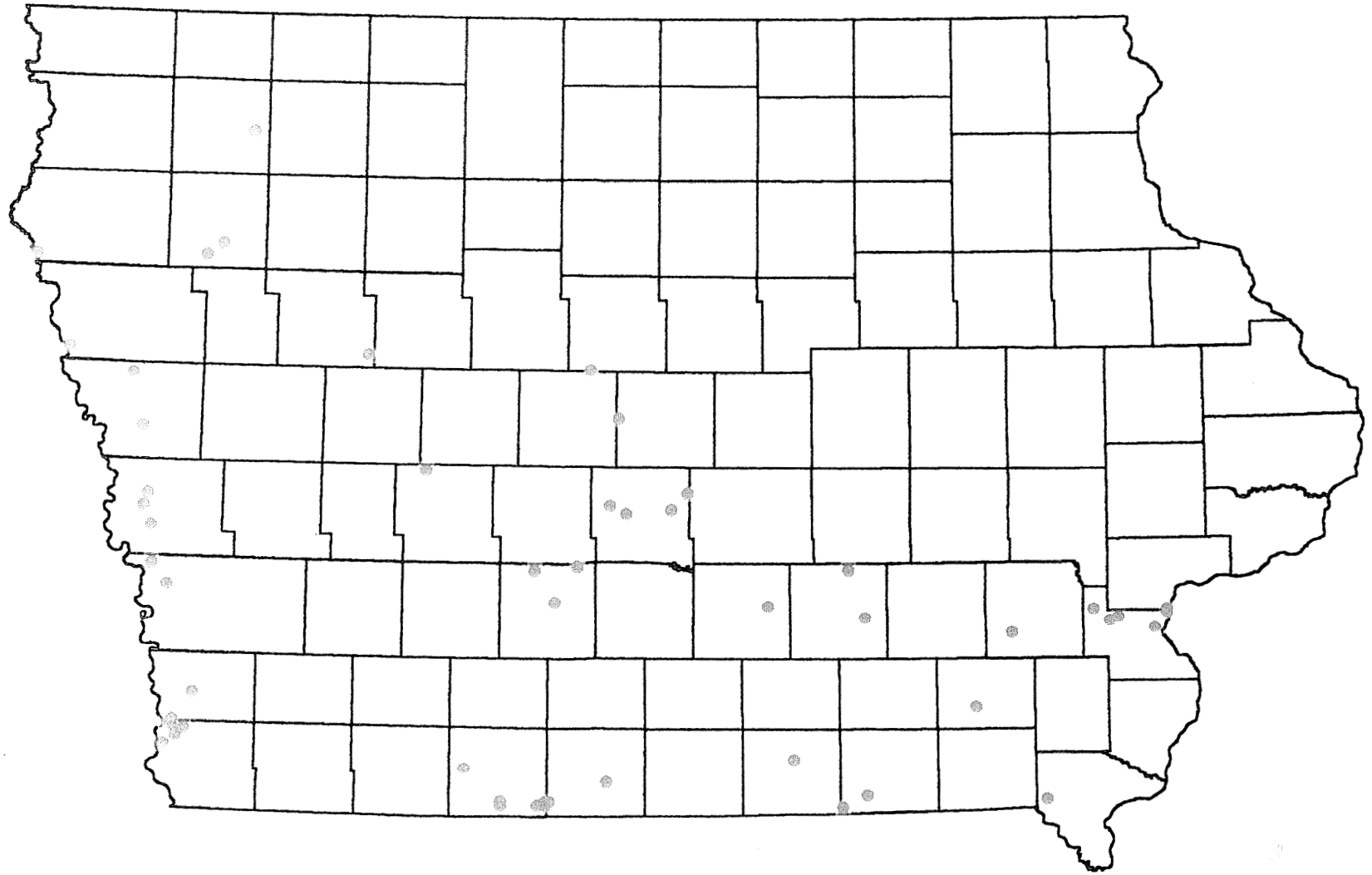

Figure 7: Distribution of *H. chrysoscelis* in Iowa, based on counts of nucleoli: specimens were collected from 1970-1996 and are available in the Drake University Research Collection.

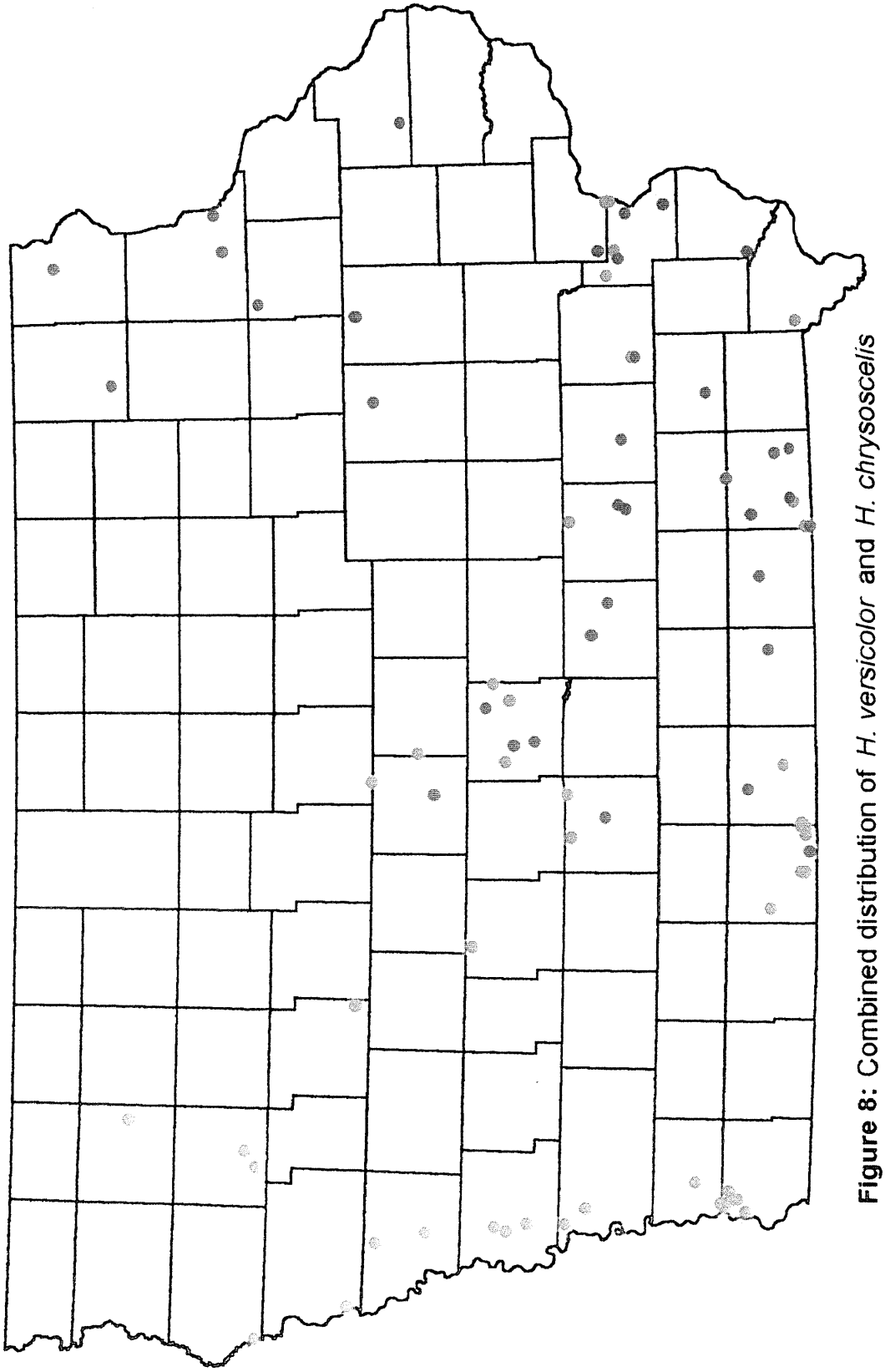

Figure 8: Combined distribution of *H. versicolor* and *H. chrysoscelis* in Iowa determined from specimens collected from 1970-1996.
● = *H. versicolor* ● = *H. chrysoscelis*

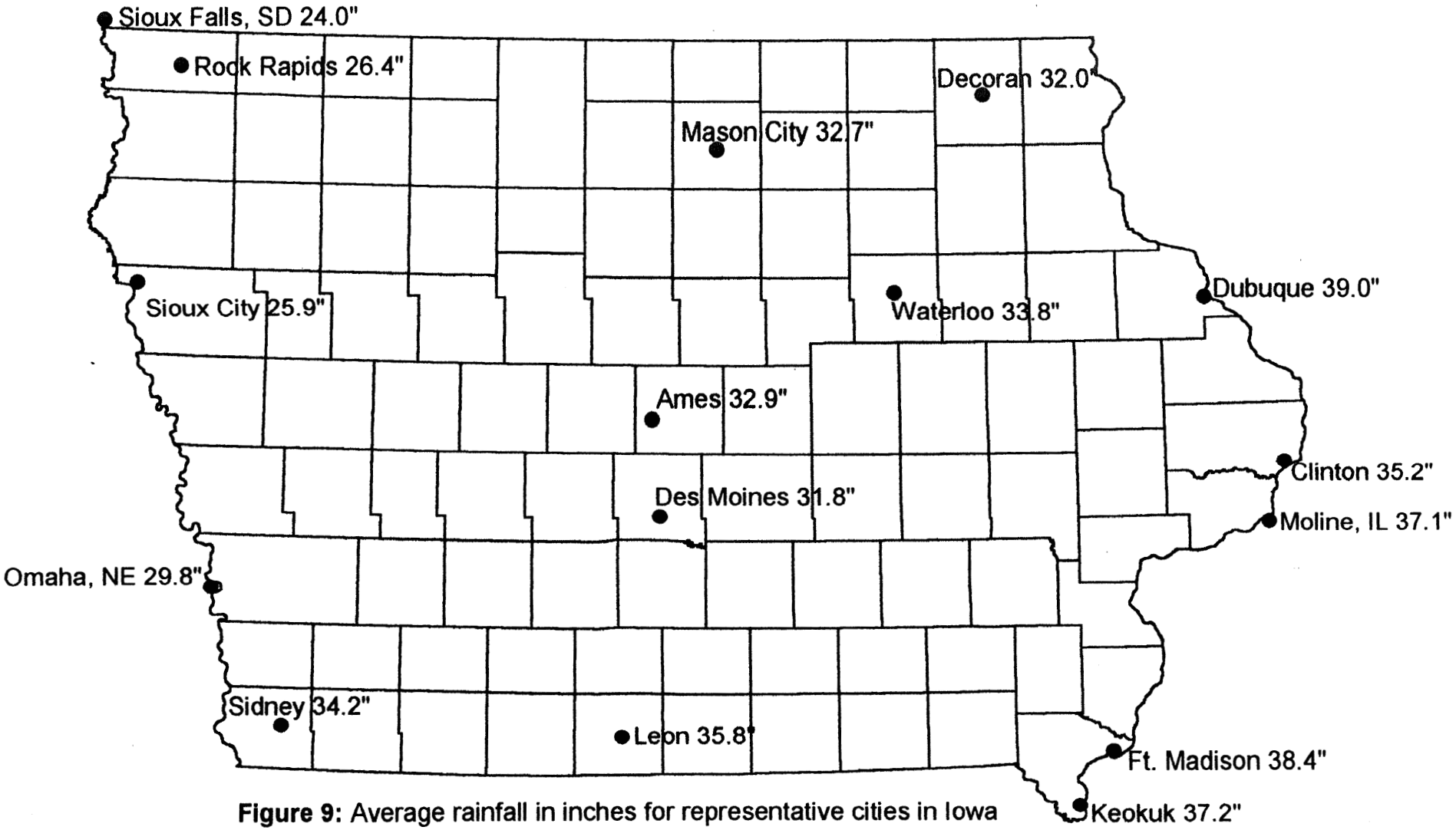

Figure 9: Average rainfall in inches for representative cities in Iowa (State of Iowa Climatologist, 1997) and surrounding states (USA Today Weather Almanac, 1995). Mean rainfall in *H. versicolor* range is 35.2"/year. Mean rainfall in *H. chrysoscelis* range is 32.9"/year.

mean rainfalls within the ranges of both species showed significant difference (P = 0.04). Geographically, *H. chrysoscelis* is found in western Iowa where greater extremes of rainfall/aridity occur and in habitats such as the Loess hills that are clearly arid. *H. versicolor* was not found in Iowa where the rainfall was less than 31.8"/year, but *H. chrysoscelis* clearly tolerated rainfall as low as 25.9"/year.

Color and spotting pattern for seventeen freshly collected specimens of *H. versicolor* and nine *H. chrysoscelis* were examined. Both species were sometimes solid green with no evidence of spotting on the back and both were sometimes gray with dark gray lichen-like markings to variations of green with gray markings. It was suspected that only *H. versicolor* would have the lichen-like markings boarded with dark black, but this too was found in *H. chrysoscelis* from Cherokee Co. Those specimens were collected and examined in the field by J. L. Christiansen. The color, large size and call suggest that the *H. chrysoscelis* from Cherokee Co. may represent a genetically different population of the species (Christiansen, personal communication).

Discussion

Past research has shown counts of nucleoli to be definitive in differentiating *H. versicolor* and *H. chrysoscelis* in states south of Iowa (Cash and Bogart, 1978; Fitzgerald, et al., 1981). For this reason, counts of nucleoli were used as the primary character in separating the 156 specimens. Of the 156 specimens examined by nucleoli staining, 65 were identified as *H. versicolor* and 91 as *H. chrysoscelis*. As seen by Cash and Bogart (1978), counts of three and four nucleoli were seen in the cells of *H. versicolor* with some cells exhibiting one or two nucleoli. The cells of *H. chrysoscelis* contained one or

two nucleoli, never more. This reflects the doubling of genetic material for *H. versicolor*. Following separation, toepad projection measurement and morphometric analysis were examined as a check for consistency of this character in distinguishing the species in Iowa. Call analysis done by Tim Armstrong was also examined as an additional check of reliability of the cellular techniques.

Toepad projection of *H. versicolor* distinguished by counts of nucleoli were larger than those of *H. chrysoscelis* (P = .016). This trend of larger toepads in *H. versicolor* supports the additional genetic material found in the tetraploid cells (Cash and Bogart, 1978; Green, 1980). Due to overlap of projection measurements, the test is only somewhat reliable as a diagnostic test. It does support differentiation of the species by counts of nucleoli. These counts provide an infallible method identification (Fitzgerald, et al., 1981).

During the 1996 mating season, call analysis by Armstrong (personal communication) found frogs at several localities, all of which agree in call analysis with conclusions drawn from counts of nucleoli. Sonograms of specimens' calls were recorded and analyzed for accurate identification. He verified identification with DNA content by measure of erythrocyte cell volume with flow cytometry (Armstrong, personal communication; Gerhardt et al., 1994). As evidenced by Figures 3 and 4, the call sonograms are distinctly different in both frequency and pulse shape. *H. versicolor* has a longer call with a slower repetition rate. *H. chrysoscelis* has a shorter call with a faster repetition rate. Figure 5 shows the distribution of the frogs analyzed by Armstrong illustrating consistency with distributional patterns shown in Fig 7.

Though no macroscopic morphological characteristics can be used to distinguish individual specimens of these species, they clearly represent two morphologically distinct populations (Table 2). Results of the t-tests show means of 11 of the 13 characteristics studied to be significantly larger in *H. versicolor*, however, ANOVA show only seven of the 13 characteristics to be significantly larger in this species. This difference is a reflection of the large overlapping variation in the morphometric measurements. Evolutionary closeness could explain the overlap (Ralin and Selander, 1979; Ptacek et.al., 1994). It is likely that using 35mm as the criteria for defining adults may have excluded several adults of *H. chrysoscelis,* the smaller species. *H. chrysoscelis* was never larger than *H. versicolor* in means for any character examined.

The mapping of the two species presented herein (Figure 8), showing *H. chrysoscelis* in the southwestern half of Iowa and *H. versicolor* in the southeastern half, follow the distribution pattern seen in Kansas by Hill et. al., (1987). Ralin (1968) observed that the species have different ecological requirement in that *H. chrysoscelis* can tolerate and may prefer lower humidity than *H. versicolor*. Iowa rainfall data shown in Figure 9 show that in Iowa, *H. chrysoscelis* tends to tolerate more arid areas such as the loess hills, and *H. versicolor* tends to be limited to the more humid Mississippi River Valley. A study relating relative humidity to calling individuals show a trend toward a greater number of *H. versicolor* calling at higher humidities and a higher number of *H. chrysoscelis* calling at lower humidities (Ralin, 1968). Ralin (1968) also reported a difference in calling position and stomach contents indicating that *H. chrysoscelis* is more arboreal than *H. versicolor*. In forests, relative humidity decreases with height

above ground. When the species live sympatrically, different ecological preferences could explain how they may avoid direct competition.

There appears to be an absence of the *H. versicolor* complex in the north central section of the state. Comparison with a map of the complex provided by Christiansen (personal communication) shows a substantial reduction in the northern two tiers of counties in Iowa. This could reflect habitat destruction due to agriculture, sampling error, or increasing UV-B radiation as well as other factors. Sampling by Christiansen and Van Gorp was intense in the northern tier counties in the course of a search for *Acris crepitans* in 1995 and 1996 (Van Gorp, 1997). The north central section of Iowa is heavily farmed and may not provide choice habitat. Historically, the area was tall grass prairie and lacked the prime habitat for treefrogs. It is also on the Wisconsin Glacial deposit and is higher and cooler than the surrounding terrain (Prior, 1991). Other studies verify the declining amphibian populations occurring in a North to South pattern which would affect this section of Iowa (Van Gorp and Van De Walle, 1995; Van Gorp, 1996; Van Gorp, 1997; Van Gorp and Christiansen, 1997).

This decline in population may stem from several sources. Alterations in habitat, such as clearing for agriculture or development, may contribute to the decline. Increased UV-B radiation may affect the viability of eggs or offspring. Disease may have a role in the decline. No link has been made to agricultural or industrial pollutants (Christiansen, personal communication).

Species DNA fingerprinting is a definitive test which could be used to determine the genetic difference between *H. versicolor* and *H. chrysoscelis*. Random Amplified

Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphisms (RFLP) and isozyme analysis are fingerprinting techniques by which genetic variability between and within species can be determined (Yu et. al.,1993; Smith and Pham, 1996). The RAPD, RFLP and isozyme analysis tools could be used not only to differentiate between the species, but could also show variation between populations (Yu et. al.,1993; Smith and Pham, 1996). RAPD analysis was attempted in this study, however, a limited number of fresh specimens prevented the collection of meaningful data.

Conclusion

The *H. versicolor* complex can be separated in Iowa on the basis of counts of nucleoli, enabling mapping of the distribution of the two species in Iowa. Results based on counts of nucleoli are supported by toepad projection measurement, call analysis and rainfall data. Morphometric analysis supports findings that suggest *H. versicolor* is a larger frog than *H. chrysoscelis*. Of the three techniques, morphology and toepad projection measurement are less definitive than counts of nucleoli. Morphological measurements overlap too much to be used as a diagnostic tool for identification of individuals but clearly show that *H. versicolor* is larger. Toepad projection size is somewhat reliable, but like other measurements is not absolute. Counts of nucleoli provide a cost-effective, definitive method of differentiation of both fresh and preserved specimens. A fourth method, DNA fingerprinting, is potentially the most definitive form of differentiation. DNA analysis could enable the comparison of evolutionary closeness between species and/or populations.

Mapping shows *H. versicolor* is present in the southeastern half of the state and *H. chrysoscelis* in the southwestern half of Iowa. This suggests an ecological tolerance for less humid conditions by *H. chrysoscelis*. The suggestion of even slightly different ecological preferences helps explain how such similar species can exist sympatrically. Sympatric populations provide an opportunity for interbreeding; however, high mortality in lab crosses and no record of hybridization shows that the species appear to be reproductively isolated. The disappearance of these frogs from many of the northern Iowa counties, is cause for concern.

It is necessary for the scientific community to know the distribution of individual species when examining the cause and extent of the declining amphibian populations. Likewise, baseline data established by this study will be a necessary component of future studies of changes in Gray Treefrog populations mapped as such.

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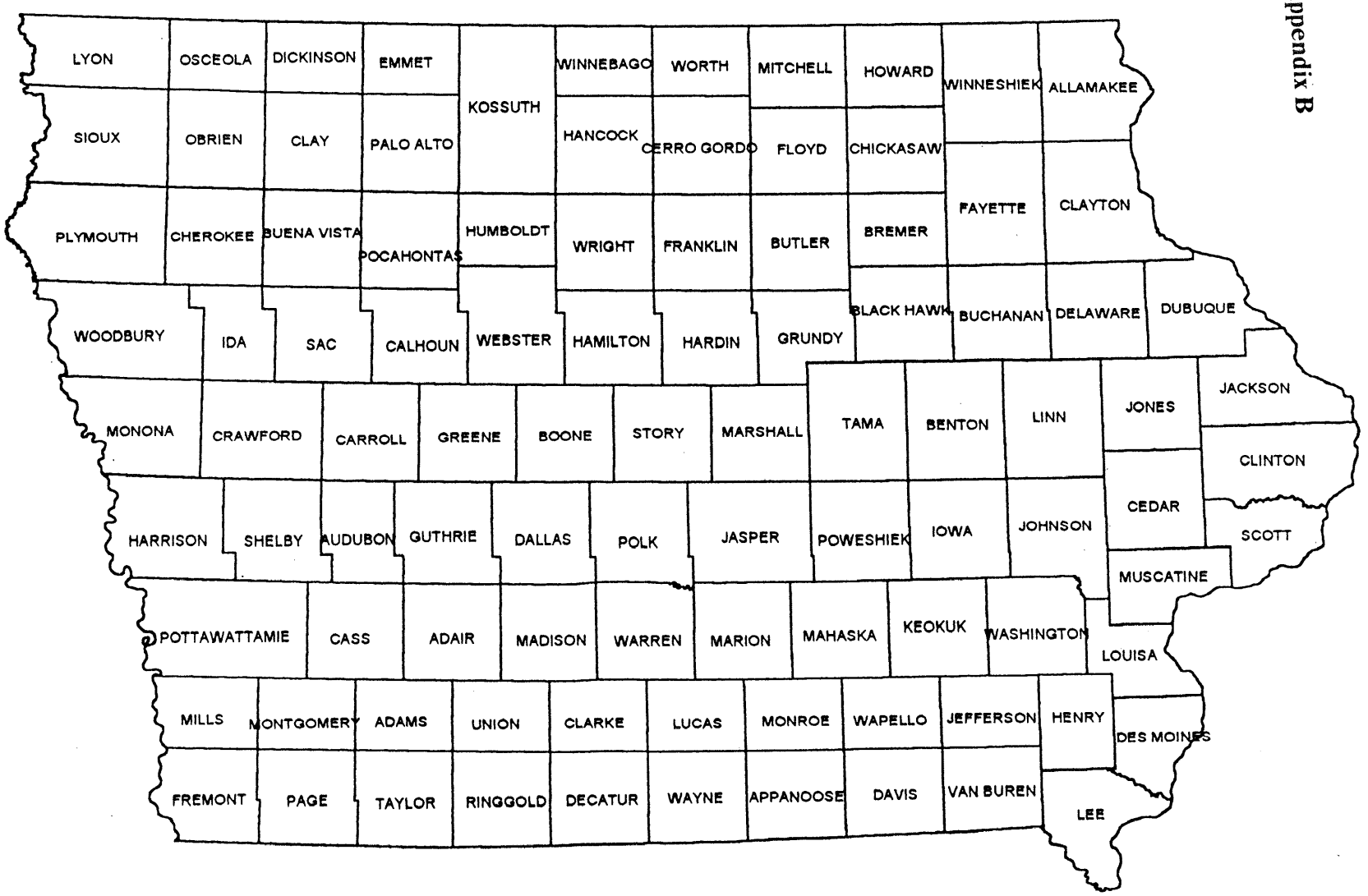
DURC#	Genus	species	collector	Date	State	County	Sect.	Twp.	Range
1527	Hyla	versicolor	J.P. Spurgeon	22-May-83	IA	Davis	20	T68N	R14W
1529	Hyla	versicolor	J.P. Spurgeon	18-Jun-83	IA	Davis	20	T68N	R12W
1530	Hyla	versicolor	J.P. Spurgeon	18-Jun-83	IA	Davis	31	T69N	R12W
1532	Hyla	versicolor	J.P. Spurgeon	24-May-83	IA	Davis	18	T67N	R15W
1533	Hyla	versicolor	J.L. Christiansen	14-May-87	IA	Decatur	28	T70N	R26W
1537	Hyla	versicolor	J.L. Christiansen	14-May-71	IA	Madison	20	T76N	R27W
1541	Hyla	versicolor	S.D. Devine	13-Jun-71	IA	Madison	20	T76N	R27W
1545	Hyla	versicolor	J.W. Larson	30-May-71	IA	Marion	30	T76N	R18W
1547	Hyla	versicolor	J.W. Larson	30-May-71	IA	Marion	30	T76N	R18W
1549	Hyla	versicolor	J.W. Larson	30-May-71	IA	Marion	30	T76N	R18W
1552	Hyla	versicolor	R. Shanaberger	30-May-71	IA	Marion	30	T76N	R18W
1553	Hyla	versicolor	R. Shanaberger	30-May-71	IA	Marion	30	T76N	R18W
1557	Hyla	versicolor	S.D. Devine	30-May-71	IA	Marion	30	T76N	R18W
1558	Hyla	versicolor	S.D. Devine	30-May-71	IA	Marion	30	T76N	R18W
1560	Hyla	versicolor	S.D. Devine	30-May-71	IA	Marion	30	T76N	R18W
1561	Hyla	versicolor	J.L. Christiansen	14-Sep-75	IA	Marion	2	T76N	R20W
1563	Hyla	versicolor	J.L. Christiansen	30-Jun-70	IA	Polk	33	T80N	R24W
1566	Hyla	versicolor	M.W. Rhiner	28-Sep-86	IA	Polk	27	T79N	R24W
1567	Hyla	versicolor	J.M. Ellefson	22-Sep-92	IA	Polk	25	T81N	R23W
1568	Hyla	versicolor	J.T. Crawford	23-May-78	IA	Des Moines	32	T70N	R4W
1569	Hyla	versicolor	J.T. Crawford	23-May-78	IA	Des Moines	32	T70N	R4W
1570	Hyla	versicolor	J.T. Crawford	23-May-78	IA	Des Moines	32	T70N	R4W
1571	Hyla	versicolor	J.L. Christiansen	31-May-87	IA	Jackson	14	T84N	R2E
1574	Hyla	versicolor	J.T. Crawford	11-May-78	IA	Jefferson	3	T71N	R10W
1577	Hyla	versicolor	J.D. Camper	1-Jun-84	IA	Linn	13	T86N	R7W
1579	Hyla	versicolor	J.P. Spurgeon	8-Jun-84	IA	Louisa	18	T75N	R4W
1580	Hyla	versicolor	J.T. Crawford	11-Jul-78	IA	Louisa	4	T75N	R2W
1582	Hyla	versicolor	J.L. Christiansen	1-Jun-92	IA	Louisa	25	T75N	R3W
1583	Hyla	versicolor	J.L. Christiansen	21-May-74	IA	Louisa	4	T75N	R2W

1584	Hyla	versicolor	J.L. Christiansen	2-Jun-76	IA	Louisa	4	T75N	R2W
1585	Hyla	versicolor	J.L. Christiansen	2-Jun-76	IA	Louisa	4	T75N	R2W
1589	Hyla	versicolor	K.K. Sutton	24-Jun-89	IA	Muscatine	21	T76N	R4W
1590	Hyla	versicolor	E.A. Baness	12-Sep-92	IA	Washington	6	T74N	R8W
1592	Hyla	versicolor	J.D. Camper	4-Sep-83	IA	Winnishiek	9	T96N	R9W
2667	Hyla	versicolor	J. LeClere	14-Jun-95	IA	Allamakee	32	T99N	R4W
2673	Hyla	versicolor	J.L. Christiansen	30-May-95	IA	Muscatine	33	T76n	R2W
2782	Hyla	versicolor	J.L. Christiansen	11-May-95	IA	Louisa	17	T73N	R2W
2783	Hyla	versicolor	J.L. Christiansen	11-May-95	IA	Louisa	17	T73N	R2W
2784	Hyla	versicolor	T.J. Van DeWalle	11-May-95	IA	Ringgold	13	T67N	R29W
2805	Hyla	versicolor	J.L. Christiansen	28-May-90	IA	Benton	4	T85N	R10W
2806	Hyla	versicolor	J.L. Christiansen	28-May-90	IA	Benton	4	T85N	R10W
4037	Hyla	versicolor	C.E. Clarida	31-May-96	IA	Boone	21	T83N	R26W
4038	Hyla	versicolor	C.E. Clarida	31-May-96	IA	Boone	21	T83N	R26W
4039	Hyla	versicolor	C.D. Van Gorp	1-Jun-96	IA	Clayton	20	T92N	R2W
4040	Hyla	versicolor	C.D. Van Gorp	1-Jun-96	IA	Clayton	20	T92N	R2W
4041	Hyla	versicolor	C.D. Van Gorp	1-Jun-96	IA	Clayton	20	T92N	R2W
4045	Hyla	versicolor	J.S. Rest	7-Sep-96	IA	Clayton	35	T92N	R4W
4047	Hyla	versicolor	C.E. Clarida	22-Jun-96	IA	Delaware	15	T90N	R6W
4048	Hyla	versicolor	C.E. Clarida	24-Jun-96	IA	Keokuk	14	T75N	R12W
4050	Hyla	versicolor	C.E. Clarida	11-Jun-96	IA	Mahaska	24	T75N	R15W
4051	Hyla	versicolor	C.E. Clarida	11-Jun-96	IA	Mahaska	7	T75N	R14W
4066	Hyla	versicolor	J.S. Rest	9-May-96	IA	Appanoose	7	T69N	R17W
4069	Hyla	versicolor	J.S. Rest	9-May-96	IA	Appanoose	7	T69N	R17W
4070	Hyla	versicolor	J.S. Rest	9-May-96	IA	Appanoose	7	T69N	R17W
4071	Hyla	versicolor	J.S. Rest	9-May-96	IA	Appanoose	7	T69N	R17W
4072	Hyla	versicolor	J.L. Christiansen	12-Jun-96	IA	Davis	34	T70N	R15W
4073	Hyla	versicolor	J.L. Christiansen	12-Jun-96	IA	Davis	34	T70N	R15W
4074	Hyla	versicolor	J.L. Christiansen	10-Apr-96	IA	Louisa	4	T75N	R2W
4076	Hyla	versicolor	J.L. Christiansen	10-Apr-96	IA	Louisa	4	T75N	R2W

4081	Hyla	versicolor	J.L. Christiansen	12-Jun-96	IA	Muscatine	35	T76N	R2W
4083	Hyla	versicolor	J.L. Christiansen	12-Jun-96	IA	Muscatine	35	T76N	R2W
4088	Hyla	versicolor	J.S. Rest	9-May-96	IA	Wayne	19	T69N	R20W
4089	Hyla	versicolor	J.S. Rest	9-May-96	IA	Wayne	19	T69N	R20W
4090	Hyla	versicolor	J.S. Rest	9-May-96	IA	Wayne	19	T69N	R20W
4091	Hyla	versicolor	J.S. Rest	9-May-96	IA	Wayne	19	T69N	R20W
1528	Hyla	chrysoscelis	J.P. Spurgeon	24-May-83	IA	Davis	7	T67N	R15W
1531	Hyla	chrysoscelis	J.P. Spurgeon	9-Jun-83	IA	Davis	30	T68N	R14W
1534	Hyla	chrysoscelis	J.T. Crawford	18-Sep-78	IA	Decatur	9	T68N	R25W
1535	Hyla	chrysoscelis	J.T. Crawford	21-Jul-78	IA	Ringgold	27	T69N	R31W
1536	Hyla	chrysoscelis	J.L. Christiansen	30-May-88	IA	Guthrie	8	T81N	R32W
1538	Hyla	chrysoscelis	K.K. Sutton	17-Sep-88	IA	Madison	5	T77N	R26W
1539	Hyla	chrysoscelis	A.J. Quinn	14-Jun-71	IA	Madison	20	T76N	R27W
1540	Hyla	chrysoscelis	S.D. Devine	13-Jun-71	IA	Madison	20	T76N	R27W
1542	Hyla	chrysoscelis	S.D. Devine	6-Jun-71	IA	Madison	9	T77N	R28W
1543	Hyla	chrysoscelis	J.L. Christiansen	28-May-91	IA	Mahaska	9	T77N	R15W
1544	Hyla	chrysoscelis	J.W. Larson	30-May-71	IA	Marion	30	T76N	R18W
1546	Hyla	chrysoscelis	J.W. Larson	30-May-71	IA	Marion	30	T76N	R18W
1548	Hyla	chrysoscelis	J.W. Larson	30-May-71	IA	Marion	30	T76N	R18W
1550	Hyla	chrysoscelis	R. Shanaberger	30-May-71	IA	Marion	30	T76N	R18W
1551	Hyla	chrysoscelis	R. Shanaberger	30-May-71	IA	Marion	30	T76N	R18W
1554	Hyla	chrysoscelis	R. Shanaberger	30-May-71	IA	Marion	30	T76N	R18W
1555	Hyla	chrysoscelis	S.D. Devine	30-May-71	IA	Marion	30	T76N	R18W
1556	Hyla	chrysoscelis	S.D. Devine	30-May-71	IA	Marion	30	T76N	R18W
1559	Hyla	chrysoscelis	S.D. Devine	30-May-71	IA	Marion	30	T76N	R18W
1562	Hyla	chrysoscelis	J.L. Christiansen	30-Jun-70	IA	Polk	33	T80N	R24W
1564	Hyla	chrysoscelis	J.L. Christiansen	30-Jun-70	IA	Polk	33	T80N	R24W
1565	Hyla	chrysoscelis	L.M. Evans	11-Sep-84	IA	Polk	29	T80N	R22W
1573	Hyla	chrysoscelis	J.T. Crawford	11-May-78	IA	Jefferson	3	T71N	R10W
1575	Hyla	chrysoscelis	J.D. Camper	10-May-84	IA	Lee	33	T68N	R7W

1576	Hyla	chrysoscelis	J.D. Camper	10-May-84	IA	Lee	33	T68N	R7W
1578	Hyla	chrysoscelis	J.P. Spurgeon	8-Jun-84	IA	Louisa	9	T75N	R4W
1581	Hyla	chrysoscelis	J.L. Christiansen	1-Jun-92	IA	Louisa	25	T75N	R3W
1586	Hyla	chrysoscelis	J.L. Christiansen	13-Jul-77	IA	Louisa	4	T75N	R2W
1587	Hyla	chrysoscelis	J.L. Christiansen	18-May-71	IA	Louisa	33	T76N	R5W
1588	Hyla	chrysoscelis	J.L. Christiansen	18-May-71	IA	Louisa	33	T76N	R5W
1591	Hyla	chrysoscelis	D.J. Bishop	12-Sep-92	IA	Washington	31	T75N	R8W
1593	Hyla	chrysoscelis	C.M. Mabry	10-Sep-82	IA	Fremont	3	T70N	R43W
1594	Hyla	chrysoscelis	J.D. Camper	25-Sep-82	IA	Fremont	23	T70N	R43W
1595	Hyla	chrysoscelis	B.L. Mabon	25-Sep-82	IA	Fremont	14	T70N	R43W
1596	Hyla	chrysoscelis	J.L. Christiansen	28-May-83	IA	Fremont	7	T70N	R42W
1597	Hyla	chrysoscelis	J.L. Christiansen	27-May-83	IA	Fremont	32	T70N	R43W
1598	Hyla	chrysoscelis	C.M. Mabry	8-Jun-82	IA	Fremont	4	T70N	R43W
1599	Hyla	chrysoscelis	C.M. Mabry	8-Jun-82	IA	Fremont	4	T70N	R43W
1600	Hyla	chrysoscelis	C.M. Mabry	31-Aug-82	IA	Harrison	30	T79N	R43W
1601	Hyla	chrysoscelis	C.M. Mabry	5-Aug-82	IA	Harrison	35	T80N	R44W
1602	Hyla	chrysoscelis	J.T. Crawford	25-Jul-78	IA	Harrison	13	T80N	R44W
1603	Hyla	chrysoscelis	J.T. Crawford	25-Jul-78	IA	Harrison		T79N	R43W
1604	Hyla	chrysoscelis	T.G. Lucas	10-Sep-82	IA	Mills	28	T72N	R42W
1605	Hyla	chrysoscelis	C.M. Mabry	10-Sep-82	IA	Mills	34	T71N	R43W
1606	Hyla	chrysoscelis	C.M. Mabry	14-Jun-82	IA	Monona	22	T83N	R44W
1607	Hyla	chrysoscelis	J.L. Christiansen	31-May-82	IA	Monona	18	T85N	R44W
1608	Hyla	chrysoscelis	C.M. Mabry	14-Jun-82	IA	Pottawattamie	5	T76N	R43W
1609	Hyla	chrysoscelis	C.M. Mabry	9-Jul-82	IA	Pottawattamie	10	T77N	R44W
1610	Hyla	chrysoscelis	J.T. Crawford	25-Jul-78	IA	Woodbury		T88N	R43W
1611	Hyla	chrysoscelis	J.P. Spurgeon	8-Jun-84	IA	Louisa	9	T75N	R4W
1612	Hyla	chrysoscelis	J.P. Spurgeon	8-Jun-84	IA	Louisa	18	T75N	R4W
1613	Hyla	chrysoscelis	J.L. Christiansen	2-Jun-76	IA	Louisa	4	T75N	R2W
1614	Hyla	chrysoscelis	J.L. Christiansen	2-Jun-76	IA	Louisa	4	T75N	R2W
2668	Hyla	chrysoscelis	J.L. Christiansen	21-May-95	IA	Cherokee	32	T91N	R40W

2669	Hyla	chrysoscelis	J.L. Christiansen	21-May-95	IA	Cherokee	32	T91N	R40W
2670	Hyla	chrysoscelis	J.L. Christiansen	21-May-95	IA	Cherokee	32	T91N	R40W
2671	Hyla	chrysoscelis	J.L. Christiansen	21-May-95	IA	Cherokee	15	T90N	R41W
2672	Hyla	chrysoscelis	J.L. Christiansen	30-May-95	IA	Muscatine	33	T76n	R2W
2780	Hyla	chrysoscelis	J.L. Christiansen	3-Jul-95	IA	Sac	14	T86N	R35W
2781	Hyla	chrysoscelis	J.L. Christiansen	3-Jul-95	IA	Sac	14	T86N	R35W
4042	Hyla	chrysoscelis	C.E. Clarida	31-May-96	IA	Hamilton	36	T86N	R26W
4043	Hyla	chrysoscelis	C.E. Clarida	31-May-96	IA	Hamilton	36	T86N	R26W
4044	Hyla	chrysoscelis	C.E. Clarida	31-May-96	IA	Story	13	T84N	R24W
4046	Hyla	chrysoscelis	N.J. Lohman	25-Sep-96	IA	Decatur	6	T67N	R27W
4049	Hyla	chrysoscelis	C.E. Clarida	11-Jun-96	IA	Mahaska	24	T75N	R15W
4052	Hyla	chrysoscelis	C.E. Clarida	6-Jun-96	IA	Polk	23	T80N	R25W
4053	Hyla	chrysoscelis	C.E. Clarida	6-Jun-96	IA	Polk	23	T80N	R25W
4054	Hyla	chrysoscelis	C.E. Clarida	6-Jun-96	IA	Polk	23	T80N	R25W
4055	Hyla	chrysoscelis	C.E. Clarida	6-Jun-96	IA	Polk	23	T80N	R25W
4056	Hyla	chrysoscelis	C.E. Clarida	6-Jun-96	IA	Polk	23	T80N	R25W
4057	Hyla	chrysoscelis	N.J. Lohman	25-Sep-96	IA	Ringgold	7	T67N	R29W
4058	Hyla	chrysoscelis	N.J. Lohman	25-Sep-96	IA	Ringgold	10	T67N	R28W
4059	Hyla	chrysoscelis	N.J. Lohman	25-Sep-96	IA	Ringgold	1	T67N	R28W
4060	Hyla	chrysoscelis	N.J. Lohman	25-Sep-96	IA	Ringgold	6	T67N	R29W
4061	Hyla	chrysoscelis	N.J. Lohman	25-Sep-96	IA	Ringgold	6	T67N	R29W
4062	Hyla	chrysoscelis	N.J. Lohman	25-Sep-96	IA	Ringgold	12	T67N	R28W
4063	Hyla	chrysoscelis	C.E. Clarida	10-Jun-96	IA	Wapello	31	T71N	R13W
4064	Hyla	chrysoscelis	C.E. Clarida	10-Jun-96	IA	Wapello	31	T71N	R13W
4065	Hyla	chrysoscelis	C.E. Clarida	10-Jun-96	IA	Woodbury	16	T86N	R47W
4067	Hyla	chrysoscelis	J.S. Rest	9-May-96	IA	Appanoose	7	T69N	R17W
4068	Hyla	chrysoscelis	J.S. Rest	9-May-96	IA	Appanoose	7	T69N	R17W
4075	Hyla	chrysoscelis	J.L. Christiansen	5-May-96	IA	Louisa	4	T75N	R2W
4077	Hyla	chrysoscelis	J.L. Christiansen	5-May-96	IA	Louisa	4	T75N	R2W
4078	Hyla	chrysoscelis	J.L. Christiansen	11-Jun-96	IA	Mahaska	7	T75N	R14W



Iowa map with county boundaries and labels.

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